

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

IN RE APPLICATION OF: Marek NARUSZEWICZ

SERIAL NO.: NEW U.S. PCT APPLICATION

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INTERNATIONAL APPLICATION NO.: PCT/SE99/01741

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FOR: REDUCTION OF OXIDATIVE STRESS FACTORS

**REQUEST FOR PRIORITY UNDER 35 U.S.C. 119  
AND THE INTERNATIONAL CONVENTION**Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

<b><u>COUNTRY</u></b>	<b><u>APPLICATION NO.</u></b>	<b><u>DAY/MONTH/YEAR</u></b>
SWEDEN	9803334-3	01 October 1998
SWEDEN	9900371-7	04 February 1999

A certified copy of the corresponding Convention application(s) was submitted to the International Bureau in PCT Application No. **PCT/SE99/01741**.

Respectfully submitted,  
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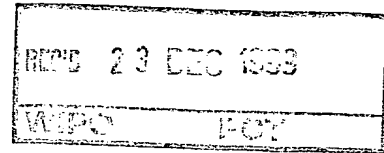
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**PRV**PATENT- OCH REGISTRERINGSVERKET  
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SE 99/1741

Härmed intygas att bifogade kopior överensstämmer med de handlingar som ursprungligen ingivits till Patent- och registreringsverket i nedannämnda ansökan.

This is to certify that the annexed is a true copy of the documents as originally filed with the Patent- and Registration Office in connection with the following patent application.

- (71) Sökande                      PROBI AB, Lund SE  
Applicant (s)
- (21) Patentansökningsnummer    9803334-3  
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Stockholm, 1999-12-13

För Patent- och registreringsverket  
For the Patent- and Registration Office

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Avgift  
Fee

**PRIORITY  
DOCUMENT**  
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## REDUCTION OF ROS

The present invention refers to the use of one or more bacterial strains to reduce the level of reactive oxygen species (ROS) in mammals including man.

## BACKGROUND OF THE INVENTION

Reactive oxygen species are produced by the monocytes and lymphocytes. Normal production of the specific level serve to maintain homeostasis in the body. A higher production of ROS is typical for acute and chronic inflammation. In chronic inflammation there is a risk of an increased aging process, atherosclerosis and cancer. Chronic inflammation patients are today treated with antibiotics, high doses of vitamins or other drugs. The use of antibiotics should for several reason be avoided and the use of drugs is mostly associated with different unwanted side-effects.

Chronic inflammatory states are for instance induced by heavy smoking or by chronic infections with viruses and bacteria. Another group of chronic inflammatory diseases comprises rheumatic diseases, which are for instance treated with the drug ibuprofen which is effective but expensive and gives gastric side-effects.

International Journal of Food Microbiology, 42 (1998) 29-38, discloses a significant increase in the total faecal concentration of the short-chain fatty acids (SCFA) acetic acid and propionic acid after 3 weeks of intake of 400 ml/d of a rose-hip drink containing oats fermented with the probiotic *Lactobacillus plantarum*. This increase, which is said to be independent from the basal diet, can either be explained by a production of SCFA by the administered probiotic strain or by said strain stimulating or suppressing other SFCA producing bacteria in the colon.

## DESCRIPTION OF THE INVENTION

~~The present invention refers to the use of a bacterial strain giving rise to increased amounts of propionic acid in the gut for the manufacture of a medicament for reduction of the level~~

of reactive oxygen species, ROS, in mammals including man. A high level of ROS is a characteristic of proinflammatory and inflammatory states, as well as high levels of the cytokines interleukin 1 and interleukin 6.

The bacterial strain is preferably a strain of *Lactobacillus* or *Propionibacterium*.

According to a preferred embodiment of the invention the bacterial strain is a *Lactobacillus plantarum* strain, such as *Lactobacillus plantarum* 299v, deposition number DSM 9843.

The invention also refers to the use of a bacterial strain giving rise to increased amounts of propionic acid in the gut for the manufacture of a medicament for the prophylaxis and/or treatment of chronic inflammatory diseases. Chronic inflammatory or proinflammatory diseases which can be treated according to the invention can be induced by different bacteria, such as *Chlamydia pneumoniae* and *Helicobacter pylori*, or toxic substances, such as nicotine. It has for instance been demonstrated that the number of antibodies against *Helicobacter pylori* was reduced after one month consumption of Pro Viva, a rose-hip drink containing oats fermented with *Lactobacillus plantarum* DSM 9843 ( $5 \times 10^7$  cfu/ml) in an amount of 400 ml/d.

A preferred use according to the invention is for the prophylaxis and/or treatment of rheumatic diseases.

## EXPERIMENTAL

### Effect of a propionic acid producing bacteria on ROS production

#### *Designation of the study*

The purpose of this study was to determine the influence of a probiotic bacterial strain on a group of six healthy volunteers with high levels of reactive oxygen species, ROS. The medium age was 32 years and the body mass index  $26.6 \text{ kg/m}^2$ . Each person was given 25 ml/d for a period of 3 weeks of a concentrated oatmeal gruel fermented with *Lactobacillus plantarum* DSM 9843 (containing  $1 \times 10^9$  cfu/ml).

#### *Method for determining Intracellular ROS production*

Blood was collected from the six individuals. Peripheral

blood mononuclear cells (PBMC) were separated by Gradisol L density gradient centrifugation.

The measurement of cell oxidation is based on reactive oxygen species (ROS) mediated conversion of nonfluorescent 2,7'-dichlorofluorescein (DCFH), loaded into cells as 2,7'-dichlorofluorescein diacetate, into fluorescent DCF with increased fluorescence emission reflecting enhanced oxidative stress. Freshly isolated PBMC, consisting of approximately 30 % monocytes and 70 % lymphocytes, were resuspended in phosphate buffered saline (PBS) followed by incubation with  $20 \mu\text{M}$  2',7'-dichlorofluorescein at  $37^\circ\text{C}$  for 30 min in the dark. The cells were then washed with PBS. The relative fluorescence intensity of the fluorophore 2',7'-dichlorofluorescein, which is formed by peroxide oxidation of its nonfluorescent precursor, was detected with cytofluorimetric analysis. During flow cytometric analysis monocytes and lymphocytes were gated on the basis of forward scatter (FCS) and side scatter (SCC). The results are expressed as mean fluorescence intensity in the following table.

Table 1.

Reactive oxygen species (ROS) production by monocytes and lymphocytes isolated from subjects before and after treatment with Pro Viva

Mean Fluorescence Intensity (counts)

Individual	Monocytes		Lymphocytes	
	Before treatment	After treatment	Before treatment	After treatment
1	220	120	86	81
2	254	369	150	134
3	562	249	217	169
4	425	201	172	135
5	343	204	188	129
6	338	224	133	106

The above data show that the production of ROS was reduced in all individuals except number 2, which is a no-responder.

#### Effect of ibuprofen on ROS production, a comparative study

In order to investigate the mechanisms behind the reduction of ROS by ibuprofen, a propionic acid derivative and a well-known antiinflammatory drug, was administered in an amount of 500 mg/d to the same group of six healthy volunteers for 3 weeks. Preliminary data indicate that the monocytes and the lymphocytes respond in exactly the same way as to the propionic acid producing bacteria in the above study.

#### CONCLUSION

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It is suggested that propionate produced in the large gut by colonic microbial fermentation may have an antiinflammatory effect. It is therefore believed that bacterial strains which give rise to increased amounts of propionic acid in the gut will decrease the proinflammatory state connected with different chronic inflammatory disorders in the body.

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## CLAIMS

1. Use of a bacterial strain giving rise to increased amounts of propionic acid in the gut for the manufacture of a medicament for reduction of the level of reactive oxygen species in mammals including man.
  2. Use according to claim 1, wherein the bacterial strain is a strain of *Lactobacillus* or *Propionibacterium*.
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3. Use according to claim 1 or 2 for the manufacture of a medicament for the prophylaxis and/or treatment of chronic inflammatory diseases.
  4. Use according to claim 3, for the prophylaxis and/or treatment of rheumatic diseases.
  5. Use according to any of claims 1-4, wherein the bacterial strain is a *Lactobacillus plantarum* strain.
  6. Use according to any of claims 1-5 of *Lactobacillus plantarum* 299v, deposition number DSM 9843.



## ABSTRACT

The invention refers to the use of a bacterial strain, preferably of the genus *Lactobacillus* or *Propionibacterium*, giving rise to increased amounts of propionic acid in the gut for the manufacture of a medicament for reduction of the level of reactive oxygen species, ROS, in mammals including man.

The medicament can be used for the prophylaxis and/or treatment of chronic inflammatory diseases such as rheumatic diseases.

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